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TITLE: Evaluation of DNA Methylation as a Target for Intraductal Therapy for Ductal Carcinoma in Situ of the Breast

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14. ABSTRACT

Ductal carcinoma in situ(DCIS), the preinvasive form of infiltrating ductal breast cancer, accounts for 20-30% of breast cancers and is treated surgically. In DCIS, the malignant cells are confined within the basement membrane, and so an ideal candidate for local therapies. Because DNA methylation is a potentially reversible mechanism for tumor suppressor gene inactivation, it is an intriguing target for molecular therapeutics. Hypothesis: DNA Methylation is altered in DCIS and is a therapeutic target for intraductal therapy. Specific Aim 1: Document the methylation status of tumor suppressor genes in DCIS. We have documented significant methylation in eight tumor suppressor genes in DCIS. Specific Aim 2: Document the feasibility of an intraductal approach to DCIS. We have successfully performed ductal lavage in 5 patients undergoing surgery for DCIS without any complications. Specific Aim 3: Identify the dose(s) of DAC with biologic activity and acceptable side effects when delivered intraductally to patients with DCIS (Phase I trial). The ultimate goal of this proposal is to evaluate DNA methylation as a target for intraductal therapy.

15. SUBJECT TERMS
Ductal Carcinoma in situ of the Breast, intraductal therapy, ductal lavage, DNA methylation, 5-aza-2'-deoxycytidine, decitidine

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Introduction: Ductal carcinoma *in situ*(DCIS), the preinvasive form of infiltrating ductal carcinoma of the breast, currently accounts for 20-30% of breast cancers and is treated by surgically removing the involved ducts. In DCIS, the malignant cells have not having invaded through the basement membrane and therefore have not gained access to the lymphatics or the systemic circulation. DCIS is a local disease, and so an ideal candidate for local therapies. DNA methylation is one mechanism for tumor suppressor gene inactivation. It is an early event in the course of malignant progression in several tumor systems. Because methylation is a potentially reversible mechanism for tumor suppressor gene inactivation, it is an intriguing target for molecular therapeutics. Drugs, such as 5-aza-deoxycytidine (DAC), are available that can reverse methylation changes and prevent tumor suppressor gene-related neoplasia *in vivo*. **Hypothesis: DNA Methylation is altered in DCIS and is a therapeutic target for intraductal therapy. Specific Aim 1:** To document the methylation status of a panel of tumor suppressor genes in DCIS. **Specific Aim 2:** Document the feasibility of an intraductal approach to DCIS. **Specific Aim 3:** Identify a dose or range of doses of DAC with biologic activity and acceptable side effects when delivered intraductally to patients with DCIS (Phase I trial). The ultimate goal of this proposal is to evaluate DNA methylation as a target for intraductal therapy. The results of this study could revolutionize the way we treat DCIS.

Body: Unfortunately due to significant administrative delays, final approval by your HSRRB was only granted on 4/14/05. As a result this report reflects work performed in the first 3 months of this study.

Task 1: a) Between 4/14/05 and 6/30/05, 5, of an expected total of 50 patients, were enrolled and ductal lavage successfully completed. There have been no complications to date. Enrollment is on target (2-3 patients per month) taking into account the delays prior to starting the study.

Task 2: Based on data generated in another study, eight of the panel of genes to be studies (APC, CALCA, cyclinD2, GSTP1, HPP1, MyoD1, RARB2, and RASSF1) were found to be hypermethylated in invasive breast cancers compared to normal breast tissues (unpublished data). Based on these findings, we have documented the methylation status of these 8 genes in 50 archival surgical specimens from patients with biopsy proven DCIS and found significant levels of methylation in all eight genes (Table 1).

Table 1: % of Samples Methylated

Gene	APC	CALCA	cyclinD2	GSTP1	HPP1	MYOD1	RARβ2	RASSF1
DCIS	57	26	61	22	39	65	78	91

We will focus on these eight genes in the ductal lavage samples.

Key Research Accomplishments:

-Final approval by HSRRB was granted on 4/14/05, representing an overall delay of 33 months to start the study.

-Identification of a subset of genes hypermethylated in breast cancer.

-Documentation of the methylation status of eight tumor suppressor genes in DCIS (Specific Aim 1)

Reportable Outcomes: Manuscript in preparation based on the data from Specific Aim 1.

Data from specific aim 1 was presented at the San Antonio Breast Cancer Symposium(12/05) in a poster entitled “Is aberrant hypermethylation and early event in breast cancer?”

Conclusions: Methylation of tumor suppressor genes is a frequent finding in DCIS. Ductal lavage can be safely performed in patients undergoing surgical treatment of DCIS.

References: N/A

Appendices: N/A